

June 30, 2021

## Control of *Hyaloperonospora brassica* by Certain Aqueous Plant Extracts in Adjara, Georgia

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### Abstract

*Cabbage (Brassica oleracea L.) is the most important vegetable among Brassica crops in the world due to its nutritional and health benefits, attacked by many harmful Pathogens. The main objective of this study was to identify and determine the main pathogens of Cabbage and their Control. Fungal cultures identification was based on macroscopic characteristics like colony morphology, colour, texture, shape and appearance were observed on potato dextrose agar (PDA) and microscopic characteristics like conidia shape, hyphen colour, concentric zone, and pigmentation. Against pathogen used 24 different plant species known for their medicinal value in traditional medicine, were cut into small pieces and dried at room temperature for one week. 19 species of fungi have been identified. 9 species of fungi involved in forming of consortium on leaves of Cabbage plants have been specified as well. Among the mycobionts found on cabbage, the most dangerous and harmful pathogen of which the dominant species is Hyaloperonospora brassicae. Among the 27 extracts evaluated, 8 extracts showed mycelia inhibition above 81.8-97.96%, 4 showed moderate effect showed non-antifungal activity against Hyaloperonospora brassicae. This may be due to the lack of antifungal compound in the above mentioned 8 extracts. Based on the overall analysis it is very clearly shown that Allium cepa, Stevia rebaudiana, Inula racemosa, Buxus colchica, Rubia coradifolia, Panax ginseng and Corylopsis sinensis extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens.*

**Keywords:** cabbage, pathogen, *Hyaloperonospora brassicae*, leaf extract, antifungal activity, inhibition effect

### INTRODUCTION

Cabbage (*Brassica oleracea*) has long been cultivated as an important vegetable crop and a source of vitamins, minerals and fibre, particularly during cold seasons in temperate climates (Lee, et al., 2015). More recently, cabbage and other cruciferous vegetables (members of the Brassicaceae) have been recognized as important sources of chemoprotective phytochemicals in the diet. Cabbage is a productive vegetable based on biomass per area of cultivation. However, this crop is affected by many diseases, particularly those caused by fungi.

In Georgia Among agricultures, Cabbage is considered one of the main cultures. The orography of the region and the edaphic - climatic conditions (frequent rain, warm humid temperature, excess of soil moisture, etc.) can forward the wide spread and development of disease-causing agents. In the first years the harvest Cabbage was considered practically painless.

Information on diseases of Cabbage is found in various works (Bérénice, et al., 2003; Bahcevandziev et al., 2015; Dickinson et al., 1977; Dickson, 1993; Göker, et al., 2009; Jensen, et al., 1999; Cogan, et al., 1998; Moss, et al., Shainidze, 2019).

The main pathogens of Cabbage cause considerable damage to the Cabbage crop especially in subtropical plains. It is increasingly becoming a limiting factor for successful cultivation of Cabbage in these regions.

June 30, 2021

Several fungicides recommended to control these pathogens not only add up to human and environmental hazards, but also have been found to be inconsistent and insufficient to deal with this disease. However, the application of this agrochemical is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Harris, et al., 2001). In many countries the use of synthetic fungicides has been banned due to polluting nature. In order to have safe methods for plant disease control in sustainable agriculture there is a need for reducing the use of synthetic chemicals fungicides there by replacing it with biocides with plant origin. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. The plants are rich sources of numerous bioactive secondary metabolites such as alkaloid, flavonoids, terpenoids, saponins, tannins and phenolic compounds which are the important sources of microbiocides, antifungal activity and many pharmaceutical compound (Mahesh, et al., 2008; Arif, et al., 2009). Plants are rich sources important organic compounds, pharmaceuticals and pesticidal compounds. Many reports on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants have reviewed. Thus plant extracts which is a source of natural pesticides can be developed into new biocidal pesticides (Samy, et al., 2000; Palombo, et al., 200; Behera, et al., 2005; Govindarajan, et al., 2006).

Therefore, this study was intended to determine the composition of pathogenic microbionts on Cabbage in all agrocenoses in Georgia; study of the symptoms, etiology and harmfulness of three fungal Cabbage pathogens and application of the biological method (various extracts of high medicinal plants) against the most common fungi *Hyaloperonospora brassicae* in Adjara, Georgia.

## MATERIALS AND METHODS

**Sample collection:** This study was conducted between 2015 June and September, 2019 in Adjara, Georgia. The objects of research were various varieties of Cabbage and pathogenic fungi inhabited on Cabbage.

**Isolation of fungi:** For isolation of fungi from *Brassica oleracea* plants, the agar method was applied. Fungal pathogens were responsible for disease isolated from fruit. From the samples obtained, serial dilutions (1:10) were prepared in test tubes with 9 ml sterile water, adding 1 g of fruit samples until dilutions 10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup>, of which 50 µL and shaken at 200 rpm for 3 hours. Samples collected were seeded by duplicate through diffusion technique on Petri dishes of 90 mm diameter containing one of the following artificial Potato Dextrose Aga (PDA) and Czapecks agar medium. After incubation (23°C for 6 days) the number for each fungus was calculated. All fungi were cultured using a single spore technique on PDA and Czapecks medium. After incubation, fungi were identified by their macroscopic and microscopic characteristics

**Identification of fungi:** Fungal cultures identification was based on macroscopic characteristics like colony morphology, color, texture, shape and appearance were observed on potatodextrose agar (PDA) and microscopic characteristics like conidia shape, hyphe color, concentric zone, and pigmentation. Species identification was based on the morphological characteristics of single – spore isolates (Foster, et al., 2004). Collections of the fungi have been examined by standard light microscopy (Pereval, Carl Zeiss, Jena and Olympus, BX50, Hamburg, Germany). The SEM micrographs have been prepared by means of a JSM-35 (Japan) SEM microscope. The specimens examined were deposited at HAL, KW and TGM (Holmgren, et al., 1990).

**Plant material:** 24 different plant species (*Alium cepa*, *Althaea officinalis*, *Betonica officinalis*, *Buxus colchica*, *Corylopsis sinensis*, *Eucalyptus cinerea*, *Galium aparine*, *Inula racemosa*, *Foeniculum vulgare*, *Hamamelis mollis*, *Hippophae rhamnoides*, *Jasminum officinale*, *Mentha sylvestris*, *Mentha longifolia*, *Panax ginseng*, *Panax ginseng*, *Pimpinella anisum*, *Pyrethrum vulgare*, *Ricinus communis*, *Rubia coradifolia*, *Rubia iberica*, *Salvia officinalis*, *Sambucus ebulus* and *Stevia rebaudiana*) known for their medicinal value in traditional medicine, were cut into small pieces and dried at room temperature for one week.

**Statistical analysis:** The data were analyzed by a one-way analysis of variance (ANOVA), and comparisons were carried out for each pair with HSD Turkey test using SPSS statistical software (SPSS Inc., Chicago, IL,

June 30, 2021

USA). All treatments were carried out in triplicate, and the values are given as means ± standard errors. Differences were considered to be significant when the P value was less than or equal to 0.05.

**RESULTS**

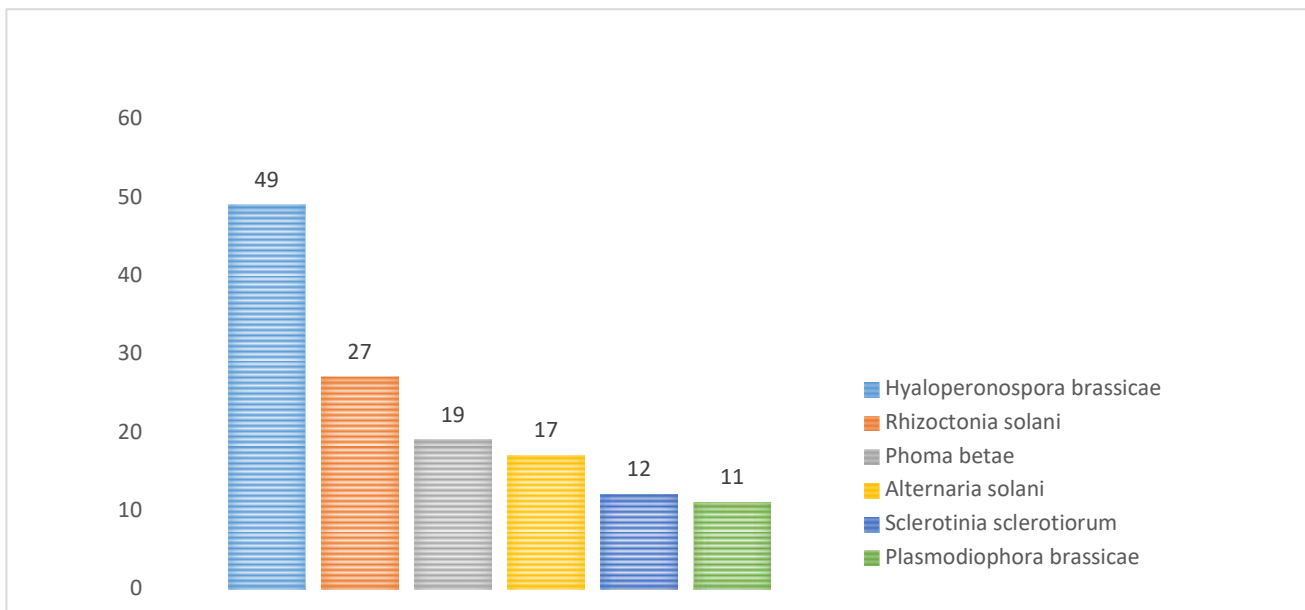
On the Cabbage, 19 species of microbionts (*Olpidium brassicae*, *Plasmodiophora brassicae*, *Hyaloperonospora brassicae*, *Phytophthora porri*, *Pythium de baryanum*, *Albugo candida*, *Mucor sp.*, *Rhizopus psapzzh*, *Erysiphe sp. brassicae*, *Penicillium sp.*, *Thielaviopsis basicola*, *Fusarium oxysporum f. sp. conglutinans*, *Fusarium moniliforme, f. sp.*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Alternaria solani*, *Alternaria sp.*, *Phoma betae*, *Rhizoctonia solani*) were identified as a result of phytopathological and mycological studies conducted in different places of agrocenosis in Georgia. Sometimes, during storage, the infected cabbage can be covered with a multi-colored mycelium which includes 9 species of fungi (*Hyaloperonospora brassicae*, *Alternaria solani*, *Alternaria sp.*, *Aspergillusniger*, *Fusariummoniliforme*, *Mucor sp.*, *Penicillium sp.*, *Sclerotiumrolfsi*, *Rhizoctonia solani*, *Rhizopus psapzzh*) in Fig. 1.

Observations have shown that the formation of the consortium begins when air and soil temperatures are 24 to - 30°C, and an optimum temperature is about 27°C. High humidity (90-95%) hastens the formation of the consortium. At present, the studies are being continued to detect the initiator fungus taking part in the formation of a consortium and to determine the relationship between the fungi taking part in it.



**Figure .1. Symptoms of consortium on cabbage leaves**

The results of the studies showed that among the mycobionts found on cabbage, the most common, dangerous and harmful are 6 patogen: *Hyaloperonospora brassicae* (49%), *Rhizoctonia solani* (27%), *Phoma betae*(19%), *Alternaria solani* (17%), *Sclerotinia sclerotiorum* (12%) and *Plasmodiophora brassicae* (11%) in Fig. 2.



June 30, 2021

**Figure 2. Spreads of the main pathogens of Cabbage in Georgia, %**

As the Fig. 2 shows fungi, the dominant species is *Hyaloperonospora brassicae* Syn.: *Peronospora brassicae*, *Peronospora parasitica*. It belongs to the Kingdom Chromista, the phylum Oomycota, and the family Peronosporaceae. It causes downy mildew. In the past, the cause of downy mildew in any plant in the family Brassicaceae was considered to be a single species *Peronospora parasitica*. However, this has recently been shown to be a complex of species with narrower host ranges, now classified in the genus *Hyaloperonospora*. From the perspective of plant pathology, *Hyaloperonospora brassicae* is now the name of the most important pathogen in this complex. Pathogens are more common in seedbeds. Under wet conditions favorable for the disease, yellow to pale brown spots develop rapidly into large irregular patches, on the upper surface of the young leaves.

A grayish white growth occurs on the underside of the leaves, and may be present, too, on the upper surface during cool and moist conditions. The infected areas turn brown and papery in dry weather. Severely affected seedlings are stunted and killed. The older leaves may have a speckled appearance in Fig. 3.



**Figure 3. Symptoms *Hyaloperonospora brassicae* in theseedbeds**

Nearly similar symptoms appear in the field and lead to early death of the leaves; in moist weather the spots grow larger, join together and form large dead patches. The infections can lead to bacterial rots developing in storage. Large dark brown rots develop on cauliflower heads.

Similar symptoms appear in the field and lead to early death of the leaves; in moist weather the spots grow larger, join together and form large dead patches in Fig. 4.



**Figure 4. Symptoms of *Hyaloperonospora brassicae* in the field on cauliflower heads**

The distribution area of Downy mildew, level of disease incidence and severity were determined during observation of Cabbage sowings in Table 1.

June 30, 2021

As shown in Table 1, the average values of infected field were high (67.6-58.5%) in subtropical zone (Xhelvachauri and Kobuleti). The lowest number of infected fields (28.6%) were in mountainous zone (Khulo). Nearly equal number of infected fields (32.4-33.1%) were determined in KedaanShuakhevi. Notwithstanding that the vegetation period of 2013 was unfavorable for Downy mildew diseases development as suitable, the highest of 2012-2016 years mean value (49.2%) of field infected by Fusarium wilt was described.

Table 1. Spread of *Hyaloperonospora brassicae* in the different geographic zones in Georgia (2015-2019)

Years	Spread of <i>Hyaloperonospora brassicae</i> in the different geographic zones (%)					Average
	Subtropical zone		Mountainous zone			
	Kobuleti	Xhelvachauri	Keda	Shuakhevi	Khulo	
2015	75.7	83.3	37.1	36.2	34.0	53.2
2016	65.6	73.0	36.6	40.0	21.0	47.2
2017	41.3	57.7	31.0	32.9	26.7	37.9
2018	73.4	78.8	38.9	37.8	39.0	53.6
2019	36.3	45.4	18.4	18.7	22.1	23.3
Average	58.5	67.6	32.4	33.1	28.6	49.2

As shown in Table 2, overall mean of incidence and severity were low (20.3% and 21.8%), especially in non-irrigated region of Shuahkevi (12.5-14.4%). Drought and hot dry weather during April and May limited development of Downy mildew and that significantly contributed to the Downy mildew development. In 2012 and 2015 years overall mean of infected fields by Downy mildew were similar (53.2% and 53.4%, respectively) and lower, than in previous years. Also, there was a very low overall mean of incidence and severity of diseases - 20.3% and 21.8% in 2013, 23.0% and 24.0% in 2014, 25.1 and 25.4 in 2015 respectively.

Low levels of disease incidence during 2014-2016 can be explained by influence of unfavorable environmental conditions. Notwithstanding that the vegetation period of 2013 was unfavorable for Downy mildew diseases development as suitable, the highest of 2012-2016 years mean value (49.2%) of field infected by Fusarium wilt was described. However, overall mean of incidence and severity were low (20.3% and 21.8%), especially in non-irrigated region of Shuahkevi (12.5-14.4%). Drought and hot dry weather during April and May limited development of Downy mildew and that significantly contributed to the Downy mildew development. In 2012 and 2015 years overall mean of infected fields by Downy mildew were similar (53.2% and 53.4%, respectively) and lower, than in previous years. Also, there was a very low overall mean of incidence and severity of diseases - 20.3% and 21.8% in 2013, 23.0% and 24.0% in 2014, 25.1 and 25.4 in 2015 respectively. Low levels of disease incidence during 2014-2016 can be explained by influence of unfavorable environmental conditions.

Different concentration i.e. 1000ppm, 3000ppm, 5000ppm of 27 different metabolic extract from 24 different medicinal plant species was tested for their efficacy against *Hyaloperonospora brassicae*. These plants were selected based on traditional medicine knowledge and random choosing from the local flora. All the extract were found to inhibit the growth of fungi. Antifungal activity of tested plant extract was investigated at different concentration against *Hyaloperonospora brassicae*. The result revealed that highly significant percent inhibition.

The antifungal activity was observed to be dose - dependent i.e. with increase in concentration of plant extract percentage inhibition of mycelium growth increases. Antifungal activity of different plant extracts showed significant activity when compared with the leaf/root extract against *Hyaloperonospora brassicae*. Root extract of *Inula racemosa* (R), *Rubia coradifolia* (R) and *Panax ginseng* (R) exhibit highest activity.

June 30, 2021

Table 2. Overall mean values of incidence and severity levels of *Hyaloperonospora brassicae*, %

Geographical zones	2012		2013		2014		2015		2016		Average	
	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %
Kobuleti	29.4	34.5	30.3	31.6	31.4	33.1	36.6	35.5	33.7	36.8	32.3	33.9
Xhelvachauri	25.5	32.8	28.3	30.8	30.2	30.7	30.5	30.6	34.7	36.2	29.8	32.2
Keda	21.3	23.4	20.1	21.8	22.9	23.8	20.0	20.9	22.5	25.5	21.4	23.1
Shuakhevi	18.4	25.0	12.5	14.4	16.5	17.1	23.8	25.0	16.8	17.9	17.6	19.9
Khulo	20.5	21.0	10.2	10.4	14.2	15.5	14.8	15.1	14.8	19.0	14.9	16.2
Average	20.0	27.3	20.3	21.8	23.0	24.0	25.14	25.42	24.5	27.08	23.2	25.1

Among the 27 extracts evaluated, 8 extract showed mycelia inhibition above 81.8-97.96%, 4 showed moderate effect of 68.3 -78.6% and 7 showed least 30.43% - 51.3% (Table 3). Remaining 8 extract from leaf extract of *Agave americana*, *Althaea officinalis*, *Betonica officinalis*, *Jasminum officinale*, *Pimpinella anisum*, *Pyrethrum vulgare*, *Ricinus communis* and *Sambucu sebulus* showed no antifungal activity against the *Hyaloperonospora brassicae*.

This may be due to lack of antifungal compound in the above mentioned 8 extracts. The percentage growth inhibition of *Hyaloperonospora brassicae* was found maximum with *Alium cepa* (97.96%), *Stevia rebaudiana* (96.78%) and *Inula racemosa* (R) (92.86%). The plant *Buxus colchica*, *Inula racemosa* (L), *Rubia coradifolia*, *Galium aparine*, *Panax ginseng* et al which inhibit the mycelia growth above 70% would probably can be an important candidates plants for prevention of against Fungi. Investigation is an important step towards crop protection strategies for antifungal activity against important phytopathogen *Hyaloperonosporabrassicae*.

Table 3. Efficacy of herbal extracts of medicinal plants against *Hyaloperonospora brassicae*

Species	1000ppm		3000 ppm		5000 ppm	
	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition
<i>Alium cepa</i> (L)	1.7±0.08	85.94±1.2	1.03±0.06	89.96±1.5	0.97±0.07	97.96±1.92
<i>Buxus colchica</i> (L)	2.2±0.07	76.45±1.4	1.4±0.07	78.85±1.4	0.86±0.06	86.63±1.84
<i>Corylopsis sinensis</i> (L)	1.9±0.09	75.5±1.1	1.02±0.05	76.45±1.6	0.78±0.08	81.7±1.56
<i>Eucalyptus cinerea</i> (L)	3.2±0.05	23.94±0.9	2.03±0.06	27.95±1.4	1.96±0.07	30.43±1.37
<i>Hamamelis mollis</i> (L)	3.1±0.08	25.83±0.8	2.09±0.09	32.86±1.55	1.97±0.05	35.5±1.24
<i>Hippopha erhamnoides</i> (L)	2.7±0.07	61.80±1.84	1.6±0.05	62.66±0.67	1.4±0.06	68.34±1.23
<i>Panax ginseng</i> (L)	1.4±0.05	70.69±1.2	1.3±0.05	73.67±1.2a	1.16±0.03	74.56±0.69
<i>Panax ginseng</i> (R)	2.5±0.04	75.84±0.7	1.3±0.04	77.82±1.22	1.16±0.05	81.80±0.72
<i>Mentha silvestris</i> (L)	3.12±0.07	30.65±1.42	3.2±0.02	31.63±0.7	2.73±0.04	37.60±0.7
<i>Mentha longifolia</i> (L)	3.4±0.04	32.3±0.8	2.9±0.05	37.6±1.37	2.3±0.04	47.9±1.13
<i>Galium aparine</i> (L)	3.3±0.07	65.37±1.1	1.75±0.04	76.34±0.82	1.52±0.04	78.32±0.7
<i>Foeniculum vulgare</i> (L)	3.05±0.06	35.82±0.6	2.52±0.02	44.26±0.62	2.2±0.05	47.44±1.3
<i>Rubia coradifolia</i> (L)	2.43±0.03	72.64±0.8	1.44±.04	76.5±0.8	1.2±0.06	78.63±1.23
<i>Rubia coradifolia</i> (R)	2.35±0.04	76.59±0.69	1.21±0.07	79.48±0.69	1.1±0.07	82.41±1.2
<i>Rubia iberica</i> (R)	3±0.02	36.18±1.25	2.2±0.05	53.13±1.22	2.1±0.05	58.27±1.27
<i>Inula racemosa</i> (L)	1.3±0.05	79.04±1.23	1.03±0.02	80.01±1.43	0.97±0.03	86.84±1.42
<i>Inula racemosa</i> (R)	1.4±0.07	82.91±1.3	1.04±0.07	88.47±1.44	0.95±0.05	92.86±1.3
<i>Salvia officinalis</i> (L)	3.7±0.08	29.52±0.5	3.5±0.06	31.52±1.5	3.04±0.06	36.12±1.5
<i>Stevia rebaudiana</i> (L)	2.37±0.04	83.74±1.3	2.03±0.08	87.76±1.7	1.7±0.08	96.78±1.7

The value means of three replicates ± standard error. The values followed by different alphabets differ significantly whensubjected to Tukey HSD at 0.5 subset; R: Root; L: Leaf.

## DISCUSSION

June 30, 2021

Bio-deterioration and spoilage of vegetables, fruits and agricultural product due to infestation by insects and microorganism can cause losses up to 100%. Several phytopathogenic fungi including *Hyaloperonospora brassicae* causes significantly decrease in nutritional value (Shainidze, 2019). *H. brassicae* has been recorded as dominant fungus in fruit and vegetables. Use of many agriculturally important synthetic fungicide against this fungus have been banned by World health organization (WHO) in several countries due to its toxicity effect against non target organisms including human. Thus, there is an urgent need to search for alternative method for prevention of bio deterioration of vegetable without any toxicity to the consumer. Plants are rich sources important organic compounds, pharmaceuticals and pesticidal compounds. Many reports on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Samy, et al., 2000; Palombo, et al., 2001; Kumaraswamy, et al., 2002; Stepanovic, et al., 2003; Bylka, et al., 2004) have reviewed.

Plant derived secondary metabolites possessing antifungal activity. There are plenty of biologically active constituent which can be used as potential sources of commercially valuable pesticides which still remain to be discovered (Balandrin, et al., 1985) This may be due to lack of information on the screening/evaluation of diverse plants for their antifungal potential. Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies.

Among the 27 extracts evaluated, 8 extract showed mycelia inhibition above 81.8-97.96%, 4 showed moderate effect of 68.3 -78.6% and 7 showed least 30.43% - 51.3% (Table 3). Remaining 8 extract from leaf extract of *Agave americana*, *Althae aofficinalis*, *Betonica officinalis*, *Jasminum officinale*, *Pimpinella anisum*, *Pyrethrum vulgare*, *Ricinus communis* and *Sambucus ebulus* showed no antifungal activity against the *Hyaloperonospora brassicae*.

This may be due to lack of antifungal compound in the above mentioned 8 extracts. The percentage growth inhibition of *Hyaloperonospora brassicae* was found maximum with *Alium cepa* (97.96%), *Stevia rebaudiana* (96.78%) and *Inula racemosa* (R) (92.86%). The plant *Buxus colchica*, *Inula racemosa* (L), *Rubia coradifolia*, *Galium aparine*, *Panax ginseng* et al which inhibit the mycelia growth above 70% would probably can be an important candidates plants for prevention of against Fungi.

Almost similar findings were made by foreign researchers (Suman et al., 2016). Investigation is an important step towards crop protection strategies for antifungal activity against important phytopathogen *Hyaloperonospora brassicae*.

## Conclusion

The study ascertains the value of plants used in traditional medicine thereby concluding that *Alium cepa*, *Stevia rebaudiana*, *Inula racemosa*, *Buxus colchica*, *Rubia coradifolia*, *Panax ginseng* and *Corylopsis sinensis* extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens.

## Acknowledgement

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**Significant Statement:** Based on a general analysis, it is very clearly shown that the investigation is an important step towards developing plant protection strategies for antifungal activity against the important phytopathogen *Hyaloperonospora brassicae*. That can be beneficial for farmers and researchers who involve in agriculture.

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June 30, 2021

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June 30, 2021

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